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Daniel A. Portnoy

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EXAMINER

MAKAR, KIMBERLY A

ART UNIT

PAPER NUMBER

1636

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
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3 MONTHS

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PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

Office Action Summary	Application No.	Applicant(s)	
	10/627,452	PORTNOY ET AL.	
	Examiner	Art Unit	
	Kimberly A. Makar, Ph.D.	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 11 January 2007.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 13-43 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 13-43 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☒ The drawing(s) filed on 25 July 2003 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____ |
| 3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date <u>01/11/07</u> . | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

Cancellation of claims 1-12 by applicant in amendment dated 1/11/2007 is acknowledged. New claims 13-43 are added and pending, drawn to a method of generating an immune response in a cell of a human (claims 13-25) and a method of generating a physiological response in a cell of a human (claims 26- 43) by the administration of a recombinant non-virulent bacterium that expressed a foreign functional cytolysin operably linked to a heterologous promoter which expresses the cytolysin in the bacterium, and a second gene encoding the foreign agent.

All rejections not repeated herein based on claims 1-12 are withdrawn in light of applicant's cancellation of claims 1-12. Applicant's arguments of rejections on claims 1-12 are rendered moot in light of the cancellation and withdrawal of rejections of claims 1-12. The following rejections are necessitated by applicant's amendment dated 1/11/2007.

For the purposes of prosecution the following is defined:

Lewin et al (Viral promoters can initiate expression of toxin genes introduced into Escherichia coli, BMC Biotechnology, 2005. 5(19):1-9) teaches that the eukaryotic CMV promoter is capable of directing expression of a reporter gene in E. coli bacteria (see page 1 and figure 1). Lewin also teaches that the eukaryotic CMV vector is capable to driving the expression of a foreign hemolysin gene in E. coli, see page 4 and figure 3). Lewin et al teaches the general misconception that "since the nucleic acid sequences characterizing eukaryotic-type and bacterial promoters are different, it is usually

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regarded improbable that promoter sequences functional in eukaryotic cells are able to direct a considerable transcription initiation in bacteria", however that a high homology between bacterial and eukaryotic RNA polymerases supports the notion that eukaryotic promoters are able to initiate transcription in *E. coli*. Thus a eukaryotic CMV promoter is capable of expressing genes in *E. coli*.

The term "cytolysin" is not clearly defined in the specification. Stedman's medical dictionary defines "cytolysin" as "[a] substance, such as an antibody, capable of dissolving or destroying cells." cytolysin. (2002). In The American Heritage Stedman's Medical Dictionary. Retrieved March 08, 2007, from [http://www.xreferplus.com/entry/2774286cytolysin. \(2002\).](http://www.xreferplus.com/entry/2774286cytolysin. (2002).)

Dorlan's Illustrated Medical dictionary defines "cytolysin" as, "[a] substance or antibody that produces cytolysis (dissolution of cells); those with specific action for a certain type of cell are named accordingly, as hemolysins, etc. Cf. lysin." cytolysin. (2003). In Dorland's Illustrated Medical Dictionary. Retrieved March 08, 2007, from [http://www.xreferplus.com/entry/4162795cytolysin. \(2003\).](http://www.xreferplus.com/entry/4162795cytolysin. (2003).)

The instant specification teaches a section broadly describing cytolysins:

The subject bacteria comprise a first gene encoding a nonsecreted foreign cytolysin operably linked to a heterologous promoter. A wide variety of foreign (i.e. not native to the microbial delivery vehicle) cytolysins may be used so long as the cytolysin is not significantly secreted by the microbe and facilitates cytosolic delivery of the foreign agent as determined by the assays described below. Exemplary cytolysins include phospholipases (see, e.g., Camilli, A., et al., *J. Exp. Med.* 173:751-754 (1991)), pore-forming toxins (e.g., an alpha-toxin), natural cytolysins of gram-positive bacteria, such as listeriolysin O (LLO, e.g. Mengaud, J., et al., *Infect. Immun.* 56:766-772 (1988) and Portnoy, et al., *Infect. Immun.* 60:2710-2717 (1992)), streptolysin O (SLO, e.g. Palmer M, et al., 1998, *Biochemistry* 37(8):2378-2383) and perfringolysin O (PFO, e.g. Rossjohn J, et al., *Cell* 89(5):685-692). Where the target cell is phagosomal, acid activated cytolysins may be advantageously used. For example, listeriolysin O exhibits greater

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pore-forming ability at mildly acidic pH (the pH conditions within the phagosome), thereby facilitating delivery of the liposome contents to the cytoplasm (see, e.g., Portnoy, et al., *Infect. Immun.* 60:2710-2717 (1992)). Furthermore, natural cytolysins are readily modified to generate mutants which are screened in the assays described below or otherwise known in the art (e.g. Awad M M, et al., *Microb Pathog.* 1997, 22(5): 275-284) desired activity modifications. In general, the screening assays measure the ability of a candidate cytolysin to confer on a bacterium the ability to render a target cell vacuole permeable to a label (e.g., a fluorescent or radioactive label) that is contained in the vacuole. In a particular example, the invention provides mutations in natural cytolysin wherein highly conserved cysteine residues (e.g., cysteine 460 in PFO, cysteine 486 in LLO) are replaced by conservative amino acid substitutions which are not subject to reduction in order to prepare oxidation/reduction-insensitive cytolysin mutants which exhibit improved lytic activity. Alternatively, mutant cytolysins are selected from naturally occurring mutants by, for example, identifying bacteria which contain cytolysins that are capable of lysing cells over a narrow pH range, preferably the pH range which occurs in phagosomes (pH 5.0-6.0), or under other conditions (e.g., ionic strength) which occur in the targeted phagosomes. Nonsecreted cytolysins may be provided by various mechanisms, e.g. absence of a functional signal sequence, a secretion incompetent microbe, such as microbes having genetic lesions (e.g. a functional signal sequence mutation), or poisoned microbes, etc.

The specification does not teach any structural requirements of a cytolysin, and from reading the specification the only functional requirement of a cytolysin is the ability to lyse a cell or pore-forming abilities. Furthermore, other enzymes such as phospholipases fulfill the requirement of cytolysin.

The term "endolysin" is not defined in the specification. The Dictionary of Medicine defines "endolysin" as a, "[s]ubstance present in cells, which kills bacteria." endolysin. (2000). In Dictionary of Medicine, Peter Collin Publishing. Retrieved March 08, 2007, from <http://www.xreferplus.com/entry/1047461endolysin>. (2000).

Applicant teaches "peptidase endolysin" – "is secreted through the membrane of the bacteria and attacks a component of the surrounding bacterial wall; see Dietrich et al. p.

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182, col. 1, line 1, where Dietrich cites Loessner et al., 1995, Mol Microbiol 16, 1231-41, abstract enclosed. Loessner explains that PLY118 is a cell wall lytic enzyme which specifically cleaves between the L-alanine and D-glutamate residues of listerial peptidoglycan." Footnote, page 11, Applicant's response, dated 1/11/07.

Without a clear definition of "cytolysin" in the specification, using the broadest reasonable interpretation of the term in light of the specification, multiple dictionaries, and applicant's response, any enzyme capable of lysing a cell fulfills the definition of "cytolysin." Using this definition, "endolysin" is synonymous with "cytolysin", as it is a lytic enzyme that lyses the cell.

The term "foreign agent" is defined as, "a nucleic acid or protein" (see abstract of the instant application, and page 3, lines 4-6 of the instant application). The specification further discloses a variety of nucleic acid based agents as, "[a] wide variety of nucleic acid-based agents may be delivered, including expression vectors, probes, primers, antisense nucleic acids, knockout/in vectors, ribozymes, etc. For example, the subject bacteria are used to deliver nucleic acids which provide templates for transcription or translation or provide modulators of transcription and/or translation by hybridizing to selected endogenous templates" (page 4, line 31- page 5 line 4 of the instant application). Furthermore, the specification teaches, "the first and second genes may be the same, i.e. the same nucleic acid encoded by the cytolysin and the foreign agent" (page 4, lines 26-27 of the instant specification). Additionally, applicant's further

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teach "that in some embodiments, an antigenic agent will suffice as a therapeutic agent" (page 1 of the 132 Declaration dated 1/11/07, and page 8 of applicant's response, dated 1/11/07). Thus for the purposes of prosecution the terms "antigenic agent" and "therapeutic agent" and "foreign agent" are used synonymously.

The specification does not define "antigen presenting cell." "Antigen presenting cell" (APC) is defined as, "a cell, such as a macrophage, a B cell or a dendritic cell, that presents processed antigenic peptides and MHC class II molecules to the T cell receptor on CD4 T cells." antigen-presenting cell. (2006). In Collins Dictionary of Medicine. Retrieved March 08, 2007, from <http://www.xreferplus.com/entry/5983109>.

Thus a macrophage is an APC that presents MHC class II molecules.

The term "attenuated" is not defined in the specification. The American Heritage Dictionary defines "attenuated" as, "[t]o make (bacteria or viruses) less virulent." attenuate. (2003). In The American Heritage® Dictionary of the English Language. Retrieved March 08, 2007, from <http://www.xreferplus.com/entry/4063193>. The specification teaches, "the bacteria are attenuated to be nonreplicative, nonintegrative into the host cell genome, and/or non-motile inter- or intra-cellularly. A wide variety of suitable means for microbial attenuation are known in the art. In another particular embodiment, the bacteria are dead or non-viable prior to endocytosis by the target cell or administration to the target organism, obviating any microbial growth or metabolism in the target cell." Thus using the broadest reasonable interpretation of "attenuated" as taught by a dictionary and in light of the specification, "attenuated" bacteria are "less virulent nonreplicative, and nonintegrative into the host cell genome."

Furthermore, "nonvirulent" is not defined in the specification. Using the definition provided by the American Heritage Dictionary for "attenuated" as, "the make (bacteria or viruses less virulent." The term "nonvirulent" is synonymous with "attenuated."

The term "pharmaceutical composition" is not defined in the specification. The only reference in the specification to "pharmaceutical composition" recites, "In vivo administration generally involves administering a pharmaceutical composition containing a therapeutically effective amount of the microbes of the invention. Generally, the therapeutically effective amount is between about 1 μ g and 100 mg/kg, preferably between about 1 μ g and 1 mg/kg. The microbes are formulated into a pharmaceutical composition by combination with an appropriate pharmaceutically acceptable excipient in accordance with routine procedures known to one of ordinary skill in the art. The microbes may be used alone or in appropriate association, as well as in combination with other pharmaceutically active compounds. The microbes may be formulated into preparations in solid, semisolid, or liquid form such as tablets, capsules, powders, granules, ointments, solutions, suppositories, and injections..." (page 7, line 26 – page 8 line 4). Thus a pharmaceutical composition comprising the microbes can be the microbes alone.

Claim Objections

1. Claims 13, 15, 20, 22-23, 26, 28, 32, 34-35, and 41 are objected to because of the following informalities: Claims 13, 26, and 41 use different spellings of the word "non-

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virulent" or "nonvirulent". It would be remedial to choose one spelling of the word for consistency. Appropriate correction is required.

2. Claims 15, 20, 22-23, 28, , 32, 34-35 use different spellings of the word "non-viable" or "nonviable". It would be remedial to choose one spelling of the word for consistency. Appropriate correction is required.

Claim Rejections - 35 USC § 112

1. The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

2. Claim 25 and 36 rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

3. Claims 25 recites the limitation "the eukaryotic cell" in claim 13. There is insufficient antecedent basis for this limitation in the claim.

4. Claims 36 recites the limitation "the eukaryotic cell" in claim 26. There is insufficient antecedent basis for this limitation in the claim.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

1. The scope of enablement rejection based on claims 11-12 (cancelled by applicant in amendment dated 1/11/07) now reads on newly added claims 12-43. The

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response to applicant's arguments are addressed at the end of the current scope of enablement rejection.

2. Claims 13-43 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of activation of B3Z T-cells *in vitro* as a result of macrophage up-take of *E. coli* bacteria genetically engineered to express a nonsecreted listeriolysin (LLO) operably linked to the tetracycline gene promoter and a second gene expressing chicken ovalbumin (OVA), does not reasonably provide enablement for a method of generating an any immune response (or physiological response) comprising the step of introducing any foreign antigenic agent (therapeutic agent) into any cell of a human comprising the administration of any non-virulent bacteria genetically engineered to encode any nonsecreted foreign cytolysin operably linked to any heterologous promoter which expresses the cytolysin in the bacterium, and any second gene, one that is other than the cytolysin. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or practice the invention commensurate in scope with these claims.

3. The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the specification coupled with information known in the art without undue experimentation (*United States v. Telectronics*, 8 USPQ2d 1217 (Fed. Cir. 1988)). Whether undue experimentation is needed is not based on a single factor but rather is a conclusion reached by weighing many factors. These factors were outlined in *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Inter.,

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1986) and again in *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988) and include the following:

4. 1) *The nature of the invention.* Applicant's claims 13-25 is a method of generating an any immune response (physiological response) comprising the step of introducing any foreign antigenic agent (therapeutic agent) into any cell of a human comprising the administration of any non-virulent bacteria genetically engineered to encode any nonsecreted foreign cytotoxin operably linked to any heterologous promoter which expresses the cytotoxin in the bacterium, and any second gene, one that is other than the cytotoxin. The claimed method reads on the generation of any immune response from the cellular (presentation of MHC class I antigens vs. MHC class II antigens? *in vitro*) to the systemic level (production of antibodies?) in a human in response to contacting the cell with genetically-engineered bacteria expressing any foreign antigenic gene and a nonsecreted foreign cytotoxin for the treatment or prevention of any disease and is not enabled for one skilled in the art to make and/or use the claimed invention in light of the specification. These claims read on a method of vaccination of a human, and in the teaching of the specification include the development and application of a HIV vaccine (see page 4, lines 19-21, and table 1).

5. 2) *State of the art.* The invention embodies nascent vaccination technologies utilizing genetically-engineered bacteria to generate an immune response in a human for the treatment of any disease using any nonsecreted foreign cytotoxin and another gene. There are few instances of *in vivo* vaccinations of genetically engineered bacteria expressing a cytotoxin and any other gene. There are no successful vaccinations

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developed for HIV. Girard et al (A review of vaccine research and development: The human immunodeficiency virus (HIV). *Vaccine*, 2006. 24:4062-4081) teaches the current state of the art in the development of HIV vaccines. He teaches "the development of an HIV vaccine faces formidable scientific challenges related to the high genetic variability of the virus, the lack of immune correlated of protection, limitations with the existing animal models and logistical problems associated with e conduct of multiple clinical trials" (see abstract). He teaches that "in spite of intense and sustained immune responses by both the humoral and cell-mediated defenses, HIV is able to resist eradication and continues depleting CD4+ T cells" (page 4065) and that vaccines directed to viral CTL, neutralizing antibody, structural and non-structural genes have been unsuccessful at providing immunity and protection from infection (page 4065, 4067-4071). Furthermore, Girard teaches that developing appropriate animal models is difficult, and that "the cellular immune responses to SIVmac during primary and chronic invention differ significantly" from humans infected with HIV-1 (page 4066) and that the monkey models used are infected with extremely large doses of virus, which do not correlate with the levels of viral exposure in humans (page 4066).

6. 3) *Unpredictability of the art*. The development of vaccinations using attenuated bacteria genetically engineered to produce foreign antigenic agents is an emerging science and unpredictable for the treatment or prevention of many diseases, including, but not limited to, HIV and HCV. Attempts to produce attenuated bacterial vaccines utilizing HIV antigens in cull culture are limited by the high number of immunizations required to elicit an immune response, the relatively low immune response, and the

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cytotoxic effects of the vaccination (Wu et al, AIDS Research and Human Retroviruses, 1997. 13(14):1187-94 whole article and Burton, PNAS, 1997. 94:10018-10023 whole article, of record 07/12/06). The problems with developing an HIV vaccine are additionally outlined in Girad et al (see above). There is nothing specifically noted in the instant specification that identifies how the claimed invention could circumvent or overcome the known problems with developing a vaccine for a virus such as HIV in humans, and the skilled artisan would have to conduct tremendous amounts of research in order to make and practice the invention.

7. 4) *Number of working examples.* The specification does not provide any working examples of the generation of an immune response from a genetically-engineered bacteria generating an immune/physiological response in a human from *in vivo* experiments. The working examples are examples of activation of B3T cells in response to the administration of *E. coli* bacteria genetically engineered to produce LLO and OVA to antigen presenting macrophages *in vitro*. The conditions for using cultured cells will vary tremendously from cell type to cell type (media, nutrients, temperature, pH etc.) but will vary even more so compared to the *in vivo* cellular environment of a human where the cells are subjected to alternate conditions (sheer stress in arterial walls vs. low pH in the stomach). Table 1 of the specification suggests that the method was used target human cells (in vivo) with successful cytosolic delivery of the agent to the target cells, including HIV. However, there is no teaching of what models these "in vivo" tests were done in (are they performed in humans? In animal models? In mouse chimeras with human cells?). Table 1 only discloses that the agent (which can be plasmids) was

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delivered to the cytosol, not that the agent is expressed in the human cell, or that an immune response or physiological response was the result of the localization of the agent in the cytosol. There are no pictures of in situ hybridizations, no immunofluorescent pictures of tissues, no immunoblots to show expression of the agent or the immune response in the specification to expand on the data found in Table 1 that teaches the requirement of the preamble and conclusion of claim 13 and 26 of "generating an immune response" and "wherein an immune response is generated" or "generating a physiological response" and "wherein a physiological response to the therapeutic agent is generated" in humans. Thus there is no evidence in the specification that would enable a skilled artisan to practice the claimed invention for using genetically-engineered bacteria with any gene and any nonsecreted cytolysin for the generation of any immune response (or physiological response) in a human.

8. 5) *Amount of direction or guidance present.* The disclosure provides no specific guidance on how to make or practice the invention *in vivo*. There is little guidance on how to determine acceptable levels of application of the invention *in vivo* to produce a desired immune response for any protective immunity against a specific antigen in humans, how often the application of the invention is required for treatments in humans, or how long expression of an immune response lasts *in vivo* in humans. One skilled in the art would be required to do further experiments in order to use and practice the claimed invention.

9. 6) *Level of skill in the art.* The level of skill is high, but given that the invention relates to areas of molecular biology and medicine that contain many problems yet

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unresolved, as well as the level of unpredictability, it must be considered that the skilled artisan would have to conduct trial and error in order to attempt to practice the claimed invention.

10. 7) *The breadth of the claims.* The claims are written broadly and read on a method of generating any immune response (or physiological response) in a human using any genetically engineered bacteria that is expressing any nonsecreted foreign cytolysin and any other gene.

11. Given the above analysis of factors which the courts have determined are critical in determining whether a claimed invention is enabled, it must be considered that the skilled artisan would have needed to conduct undue and excessive experimentation in order to practice the claimed invention.

12. It is noted that this Office Action contains rejections of the same claims under 35 USC 112, 1st (enablement) and 35 USC 103 (a). While these rejections may seem contradictory, they are not because each is based upon a different legal analysis, i.e. sufficiency of the disclosure of the instant application to support claims under 35 USC 112, 1st paragraph vs. sufficiency of a prior art disclosure to anticipate or render obvious an embodiment(s) of the claimed invention (See *In re Hafner*, 161 USPQ 783 (CCPA 1969)).

Response to arguments

13. In the response dated 1/11/07, applicant argues that the application provides ample teaching to enable one of ordinary skill in this art to practice the claimed method without undue experimentation. As evidence, applicant states:

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14. The Specification teaches the introduction of a variety of foreign agents for a variety of purposes (e.g. p.4, line 19- p.6 line 3), including antigenic and therapeutic agents. The Specification teaches use of a variety of nonvirulent bacteria (e.g. p.6 lines 4-20) and target cells (e.g.p.6, line 21- p.7, line 12). The specification teaches a variety of effective routes of in vivo and ex vivo administration depending on the nature of the foreign agent (e.g. p.7, line 13- p.8, line 14). In addition, the specification provides numerous demonstrative examples of the method as applied to numerous foreign therapeutic agents (note that in some embodiments, an antigenic agent will suffice as a therapeutic agent; therapeutic agents include prophylactics such as immunizations, e.g. p.4, lines 21-22) and a variety of target cells in vivo and ex vivo (e.g. p.8, line 15- p. 10, line 11).

15. The application provides ample teaching to enable one of ordinary skill in this art to practice the claimed method without undue experimentation, persons skilled in the art have implemented the claimed method to generate an anti-tumour response and to deliver antigens in vivo (e.g. Radford et al, Gene Therapy, 2002, 9, 1455-63; Bouwer et al. PNAS 2006, 103, 5102-7; both attached).

16. For good measure, we provide herewith affirmative evidence in the form of an expert declaration averring to the foregoing. Accordingly, the uncontroverted evidence of record demonstrates that the application provides ample teaching to enable one of ordinary skill in this art to practice the claimed method without undue experimentation. Page 8, applicant response dated 01/11/07.

17. Applicant's arguments filed 01/11/07 have been fully considered but they are not persuasive. New claims 13-43 are drawn to immune responses and physiological responses in human cells, which reads on immune and physiological response in humans, especially in light of the instant specification which teaches the in vivo administration (page 7 line 13 through page 8 lines14) and the development of an HIV vaccine (see page 4, lines 19-21, and table 1). The specification does not teach working examples of performing the method in humans.

Applicant's point to p.4, lines 21-22 as evidence that the "the specification provides numerous demonstrative examples of the method as applied to numerous foreign therapeutic agents." Page 4, lines 21-22 of the instant application recite:

The bacteria also comprise a second gene encoding a foreign agent different from the cytolysin, and the subject methods may be used to deliver a wide variety of such foreign agents for a variety of applications, including diagnosis, therapy including prophylactics such as immunizations (see, e.g. HIV vaccine, Table 1) and treatments

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such as gene therapy (especially of single gene disorders amenable to localized treatment, see Table 1, below), biosynthesis, etc.; essentially any agent that the microbial host can be engineered to produce.~ In a particular embodiment, the agent is largely retained by the microbe until lysis within the target cell vacuole.

These lines seem to point to table 1, rather than provide "numerous demonstrative examples."

18. Applicants also point to examples of target cells *in vivo* and *ex vivo* which includes human target cells located on page 8, line 15 through page 10, line 11, as evidence for the targeting of human cells. These pages comprise Table 1, which the specification teaches represents "analogous studies conducted in a variety of animals and animal cell types, both *in vivo* and *in vitro*, using a variety of agents, secretory deficient cytolysins, bacterial types and methods demonstrate consistent delivery of the agent to the target cell cytosol, as measured by agent activity, immunoassay or other delivery monitoring assays described herein" (page 8, lines 24-27).

19. However, the data represented in Table 1 is unclear. The table appears to teach administration methods which include "in situ", "intratumor injection" "in vivo" "direct injection" "intraocular injection" "IV injection" and "oral, opical abrasion" as methods of administration to human cells. The examiner is unable to find any other detail of these methods of Table 1 in the specification. The examiner is unable to find a definition of these terms in the specification. What does "in situ" mean? Are these methods performed on/in humans? How many humans? How many applications of these methods were required to generate the immune or physiological response? What was the immune response? Was it a systemic response? A cellular response? Was it sustained for a period of time? Or are these methods performed in animal models (chimeras comprising human cells) or transgenic models that express human markers?

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Or immuno-supressed animals that have been injected with human hybridomas or cell lines? It is unclear from the specification what this table is actually relaying. In light of the newly added claims specifically directed to humans and human cells, is the examiner to understand this table is a reflection of the method in which a human with a Hepatitis C infection had a direct injection of "S. typhimurium, attenuated, invasion/autolysin" comprising a non secreted LLO and the foreign agent Hepatitis C virus-specific ribozyme directly injected in their hepatic cells, as suggested by table 1, page 10, second line? Is that what the following lines adapted from Table 1 are relaying?

The only portions of Table 1 relating to apparent *in vivo* applications on humans cells are listed below:

<u>Target Cell</u>	<u>Indication</u>	<u>Agent</u>	<u>Lysin</u>	<u>Bacteria</u>	<u>Administration</u>	<u>Cytosol Delivery</u>
human prostate	localized prostatic carcinoma	ribozyme or antisense against CDK 2 or CDK4	LLO	S. typhimurium, attenuated invasion/autolysin	in situ; intratumor injection	+++
human hepatic cells	Hepatitis C infection	Hepatitis C virus-specific ribozyme	LLO	S. typhimurium, attenuated, invasion/autolysin	in vivo; direct injection	+++
human hepatic cells	diabetes	insulin receptor expression construct	LLO	S. typhimurium, attenuated, invasion/autolysin	in vivo; direct injection	+++
human beta islet cells	diabetes	insulin expression construct	LLO	S. typhimurium, attenuated, invasion/autolysin	in vivo; direct injection	+++
human cytotoxic T-cells	melanoma	melanosomal proteins	LLO	E.coli, JM109 (DE3)	in vivo; IV injection	+++
human epithelium	Herpes infection	antisense RNaseP construct	LLO	S. typhimurium, attenuated, invasion/autolysin	in vivo; oral, topical abrasion	+++

20. All other working examples in the specification are directed to *in vitro* works, as outlined in the scope of enablement rejections above. A review of the literature shows no human administration of these particular cells either.

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21. Additionally, in light of the unpredictability of the art, as taught by Grillot-Courvalin who teaches that successful transfer of the DNA into cells does not correlate with the initial number of internalized bacteria and that gene therapy utilizing attenuated bacterial vectors (i.e. bacteria harboring expression plasmids for the purpose of transmitting foreign genetic or protein material or to a hosts' cell for the generation of a physiological response) is unpredictable in regards to transformation efficiency, cell targeting, and transgene expression (see above), as well as the teaching of Girard on the problems and challenges associated with the development of an HIV vaccine, Table 1 is insufficient to teach that an immune response or physiological response results from the cytosolic delivery of the bacteria and/or vectors. There is no teaching that an immune response or a physiological response was generated. The specification fails to further clarify or expand on the data presented in table 1.

22. Applicant also points to Radford et al, Gene therapy 2002, 9, 1455-63 and Bouwer et al, PNAS 2006, 1003, 5102-7, as examples that persons skilled in the art have implemented the claimed method to generate an anti-tumor response and to deliver antigens in vivo. This argument is respectfully not persuasive. The two references noted comprise the administration of the attenuated bacteria expressing nonsecreted cytolytins in mice. There is no evidence of record to show that the mouse models used in these references are art recognized as being predictive of results which would be expected in humans. The newly drawn claims are to human cells, and read on a method performed on humans eliciting an immune response or physiological response in a human.

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23. Applicants are encouraged to provide more detail regarding Table 1 in order to overcome the scope of enablement rejections, in order to overcome the limitation reading on human cells that reads on an immune response or physiological response in a human.

24. The declaration under 37 CFR 1.132 filed 01/11/07 is insufficient to overcome the rejection of claims 1-12 (now cancelled) based upon 112 1st paragraph scope of enablement rejections as set forth in the last Office action, and which now reads on claims 13-43 (newly added) because: the newly added claims narrow the method claims of 13-43 to humans, and this limitation is not addressed in the 132 declaration.

25. The 132 Declaration, points to two peer reviewed journal articles, Radford et al (Gene Therapy 2002, 9, 1455-63) and Bouwer et al (PNAS, 2006, 103, 5102-7) as evidence that "persons skilled in the art have implemented the claimed method to generate an anti-tumour response and to deliver antigens in vivo" (page 1 or the 37CFR1.132 Declaration. However, these two articles, of record 01/11/2007, fail to disclose the generation of "an immune response" or a "physiological response" in a human or human cell. Both of these references disclose the methodologies are directed and performed in mice. Radford et al teaches the direct subcutaneous injection of the recombinant bacteria into C57Bl/6 and Tap1-/- mice (see page 1458 and figure 4). Bouwer et al teaches the direct injection of the recombinant bacteria into BALB/c and immuno-suppressed SCID mice (see page 5103). Neither of these documents disclose the generation of an "immune response" or "physiological response" in a human.

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26. The 132 Declaration also points to sections of the specification which allegedly supports the enablement of the claimed invention. The declaration states:

27. The Specification teaches the introduction of a variety of foreign agents for a variety of purposes (e.g. p.4, line 19- p.6 line 3), including antigenic and therapeutic agents. The Specification teaches use of a variety of nonvirulent bacteria (e.g. p.6 lines 4-20) and target cells (e.g.p.6, line 21- p.7, line 12). The specification teaches a variety of effective routes of in vivo and ex vivo administration depending on the nature of the foreign agent (e.g. p.7, line 13- p.8, line 14). In addition, the specification provides numerous demonstrative examples of the method as applied to numerous foreign therapeutic agents (note that in some embodiments, an antigenic agent will suffice as a therapeutic agent; therapeutic agents include prophylactics such as immunizations, e.g. p.4, lines 21-22) and a variety of target cells in vivo and ex vivo (e.g. p.8, line 15- p. 10, line 11).

28. Of the above mentioned sections of the specification that supports the enablement of the method to generate an "immune response" or a physiological response" in humans or human cells, the only evidence directed to in vivo administration is suggested by p. 8, line 15- p. 10 line 11. These pages encompass Table 1. However, Table 1 is unclear and does not fully overcome the enablement rejection on the cancelled claims or newly added claims now directed toward humans.

29. The table appears to teach administration methods which include "in situ", "intratumor injection" "in vivo" "direct injection" "intraocular injection" "IV injection" and "oral, opical abrasion" as methods of administration to human cells. The examiner is unable to find any other detail of these methods of Table 1 in the specification. The examiner is unable to find a definition of these terms in the specification. What does "in situ" mean? Are these methods performed on/in humans? How many humans? How many applications of these methods were required to generate the immune or physiological response? Or are these methods performed in animal models (chimeras comprising human cells) or transgenic models that express human markers? Or

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immuno-supressed animals that have been injected with human hybridomas or cell lines? It is unclear from the specification what this table is actually relaying. In light of the newly added claims directed to humans and human cells, is the examiner to understand this table is a reflection of the method in which a human with a Hepatitis C infection had a direct injection of "S. typhimurium, attenuated, invasion/autolysin" comprising a non secreted LLO and the foreign agent Hepatitis C virus-specific ribozyme directly injected in their hepatic cells, as suggested by table 1, page 10, second line? Is that what the following lines adapted from Table 1 are relaying?

The only portions of Table 1 relating to apparent *in vivo* applications on humans cells are listed below:

<u>Target Cell</u>	<u>Indication</u>	<u>Agent</u>	<u>Lysin</u>	<u>Bacteria</u>	<u>Administration</u>	<u>Cytosol Delivery</u>
human prostate	localized prostatic carcinoma	ribozyme or antisense against CDK 2 or CDK4	LLO	S. typhimurium, attenuated invasion/autolysin	in situ; intratumor injection	+++
human hepatic cells	Hepatitis C infection	Hepatitis C virus-specific ribozyme	LLO	S. typhimurium, attenuated, invasion/autolysin	in vivo; direct injection	+++
human hepatic cells	diabetes	insulin receptor expression construct	LLO	S. typhimurium, attenuated, invasion/autolysin	in vivo; direct injection	+++
human beta islet cells	diabetes	insulin expression construct	LLO	S. typhimurium, attenuated, invasion/autolysin	in vivo; direct injection	+++
human cytotoxic T-cells	melanoma	melanosomal proteins	LLO	E.coli, JM109 (DE3)	in vivo; IV injection	+++
human epithelium	Herpes infection	antisense RNaseP construct	LLO	S. typhimurium, attenuated, invasion/autolysin	in vivo; oral, topical abrasion	+++

However, there is no disclosure of working examples in the specification that would garner further insight into the results of the cytosolic delivery of the antigens of the "in vivo" application to human cells disclosed by Table 1. Without further clarification of the data from Table 1, this table, the only area of evidence to support the newly claimed methods reading on *in vivo* treatment of humans, this data does not provide enough

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teaching that one skilled in the art would be able to make and use the invention without undue experimentation.

Claim Rejections - 35 USC § 103

30. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

31. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

32. Claims 13-43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Darji et al (Cell, 1997. 91:765-775) in view of Powell et al (US Patent No 5,877,159) and Darji et al (Journal of Biotechnology, 1995) and Dietrich et al (Nature Biotechnology, 1998) (all of record 07/12/06). Claims 13-43 recite a method of generation an immune response comprising the step of introducing a foreign antigenic agent into a cell of a human by contacting the cell with a non-virulent bacterium comprising a first gene

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encoding a nonsecreted foreign functional cytolysin operably linked to a heterologous promoter which expresses the cytolysin in the bacterium, and a second gene encoding the foreign antigenic agent under conditions whereby the agent enters the cell, wherein an immune response is generated (claim 13). The method is further limited wherein the bacterium is endocytosed into a vacuole of the cell, the bacterium undergoes lysis and the cytolysin mediates transfer of the agent from the vacuole to the cytosol of the cell (claim 14) and wherein the bacterium is dead or non-viable (claim 15). The method is further limited wherein the bacterium comprises the cytolysin (claim 16), wherein the agent is synthesized by the bacterium (claim 17), wherein the bacterium is engineered to deliver to antigen-presenting cells antigenic polypeptides which are present in association with MHC proteins (claim 18). The method is further limited wherein the bacterium is nonreplicative and nonintegrative into the host cell genome (claim 19). The method is further limited wherein the bacterium is a dead or non-viable laboratory strain of *E. coli* (claim 21) and the bacterium comprises the cytolysin (claim 21 and 22), and the cytolysin is listeriolysin (claim 23). The method is further limited wherein the bacterium is a laboratory strain of *E. coli*, engineered to deliver to antigen-presenting cells antigenic polypeptides which are presented in association with MHC proteins (claim 24), and wherein there is no growth or metabolism of the bacterium in the eukaryotic cells.

33. Claim 26 recites a method of generating a physiological response comprising the step of introducing a foreign therapeutic agent into a cell of a human by contacting the cell with a non-virulent bacterium comprising a first gene encoding a nonsecreted

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foreign function cytolysin operably linked to a heterologous promoter which expresses the cytolysin in the bacterium, and a second gene encoding a foreign therapeutic agent, different than the cytolysin, under conditions whereby the therapeutic agent enters the cell, wherein a physiological response to the therapeutic agent is generated. The method is further limited wherein the bacterium is endocytosed into a vacuole of the cell, the bacterium undergoes lysis and the cytolysin mediates transfer of the agent from the vacuole to the cytosol of the cell (claim 27) and wherein the bacterium is dead or non-viable (claim 28) The method is further limited wherein the bacterium comprises the cytolysin (claim 29), wherein the agent is synthesized by the bacterium (claim 30) and wherein the bacterium is nonreplicative and nonintegrative into the host cell genome (claim 31). The method is further limited wherein the bacterium is a dead or non-viable laboratory strain of E. coli (claim 32) and the bacterium comprises the cytolysin (claim 33 and 34), and the cytolysin is listeriolysin (claim 35) and wherein there is no growth or metabolism of the bacterium in the human cell (claim 36). The method is further limited wherein the therapeutic agent is selected from an antibiotic, insecticide, fungicide, antiviral agent, anti-protozoan agent, enzyme, anti-cancer agent, antibody, anti-inflammatory peptide, and transcription factor (claim 37), and the method is indicated by a disease selected from the group consisting of cancer, infection, degenerative disease, and diabetes (claim 38). The method is further limited wherein the cell is a leukocyte (claim 39) or a tumor cell (claim 40). The method is further limited wherein the contacting step comprises administering a pharmaceutical composition comprising a

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therapeutically effective amount of the nonvirulent bacterium (claim 41), and wherein the administration is in vivo (claim 42) or ex vivo (claim 43).

34. Darji teaches the oral vaccination of mice using attenuated (nonvirulent) *Salmonella typhimurium* bacteria containing recombinant vectors expressing a truncated portion (amino acids 26-482) of listeriolysin protein (i.e. a foreign functional nonsecreted cytolysin) and the membrane protein ActA (i.e. a foreign antigenic agent/therapeutic agent) driven by the heterologous promoter CMV (page 766, column 1, Results Section). The CMV promoter is capable of driving expression of the listeriolysin and ActA in bacteria, as eukaryotic CMV promoters drive expression in *E.coli* (see above). The attenuated (ie nonreplicative and noninvasive) *Salmonella* are delivered as a pharmaceutical composition comprising sodium bicarbonate buffer containing the recombinant *Salmonella* (page 773).

35. Applicants do not define "functional nonsecreted cytolysin." From reading the specification, the "functional" aspect of a cytolysin is the cytolysin's ability to lyse a cell and/or pore-forming abilities. Darji teaches that his system "exploits the pore-forming activity of listeriolysin. This activity of listeriolysin allows the introduction of soluble passenger proteins into the cytosol of target cells" (page 766). Thus Darji teaches that amino acids 26-482 are functional pore-forming fragments of cytolysins which are expressed in the bacteria.

36. Darji teaches that a specific immune response (a physiological response) is detected in mice after exposure to bacteria expressing the nonsecreted LLO and the ActA (page 766, column II, lines 17-25, and Figure 1). Darji further teaches in vitro (ex

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vivo) experiments in mouse primary macrophages that demonstrates an efficient transfer of plasmid DNA (foreign agent/therapeutic agent) from attenuated bacteria into the nucleus of the phagocytic host cells which stimulates an immune response (page 766, column I). Macrophages are MHC presenting cells (see above). The specification also discloses that macrophages are leukocytes (page 6, lines 25). Darji teaches the macrophages are incubated with gentamycin and tetracycline, in order to kill any extracellular bacteria, and kill any viable intracellular bacteria (page 769, column II), thereby preventing growth or metabolism of the bacterium in the cell.

37. Darji teaches that the vaccines are developed with the intention of further use in humans, and states, "[s]afe attenuated strains of Salmonella are available and are already in use as vaccine in man and farm animal...finally, recombinant plasmids constructed in laboratory strains of E. coli can be directly introduced into salmonellae without further manipulation (page 765, column II). Darji teaches that attenuated bacteria of Shigella and E. coli, are capable of escaping the phagolysosome into the cytosol, "it follows a that lysis of bacteria in this compartment would allow transfer of bacteria in this compartment would allow transfer of the released plasmid DNA into the nucleus" (page 770, Discussion section) and his attenuated Salmonella die in the phagocytic compartment of the cells which liberates the plasmid (therapeutic/foreign antigenic agent) based eukaryotic expression vectors and the proteins expressed in the bacteria as a result of the eukaryotic expression vector CMV promoter (foreign antigenic agent) (page 771, column II, last paragraph). Thus there is no growth of the bacterium in the host cell, but the bacterium are able to produce the peptides before dying. Darji

further teaches that there is the possibility of the expression vectors expressing the proteins themselves, and performs experiments determine if the plasmids are transferred and if the immune response is due to leaky promoter expression both in vivo and in vitro (see page 768, last paragraph – page 770). Thus Darji teaches a method of generating an immune response (or physiological response) comprising the step of introducing a foreign antigenic agent into a cell by contacting the cell with an attenuated (non-virulent) bacterium comprising a first gene encoding a nonsecreted listeriolysin capable of creating pore-forming holes, operably linked to a heterologous CMV promoter, inherently capable of driving expression of the listeriolysin in the bacterium, and a second gene encoding a foreign antigenic agent (ActA) under conditions whereby the agent enters the cell and an immune response is generated. Darji does not teach that the vaccination occurs in humans or in human cells; nor, that the method is performed in a laboratory strain of E. coil.

38. Powell et al (US Patent No. 5,877,159) teaches live bacterial vector vaccines (see abstract). Powell states, "these vaccines can enter the host, either orally, intranasally or parenterally. Only gaining access to the host the bacterial vector vaccines express an engineered prokaryotic expression cassette contained there that encodes a foreign antigen(s). ...delivery of the foreign antigen to the host tissue using bacterial vector vaccines results in host immune responses against foreign antigen, which provide protect against the foreign antigen originates (column 1, lines 37-41, lines 65 through column 2, line 1). Powell teaches that "invasive bacteria" are "bacteria that are capable of delivering eukaryotic expression cassettes to animal cells or animal

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tissues. "Invasive bacteria " include bacteria that are naturally capable of entering the cytoplasm or nucleus of animal cells, as well as bacteria that are genetically engineered to enter the cytoplasm or nucleus of animal cells or cells in animal tissue" (column 8, lines 36-43). Powell teaches that the same eukaryotic expression cassettes are combined with prokaryotic expression cassettes for bacterial expression (column 16, lines 31-35). Powell teaches the use of the eukaryotic CMV promoter to drive expression of the antigen (column 15, lines 42-47), and this promoter has been shown to have inherent prokaryotic expression in bacteria (see above). Powell teaches that examples of such bacteria include attenuated (nonvirulent) E. coli (column 10, lines 25-34) that are engineered by inserting genes that enable them to access the cytoplasm of an animal cells. Powell states, "examples of such genes include the invasive proteins of Shigella, hemolysin or the invasion plasmids of Escherichia, or listeriolysin O of Listeria, as such techniques are known to result in strains that are capable of entering the cytoplasm of infected animal cells (column 10, lines 45-51).

39. Powell also teaches that the invasive bacteria are used to delivery a "therapeutic agent" to animal cells or tissues. He states, for example, the eukaryotic expression cassettes can encode tumor-specific, transplant or autoimmune antigens or parts there of. Alternatively the eukaryotic expression cassettes can encode synthetic genes, which encode tumor-specific, transplant, or autoimmune antigens or parts thereof. (column 18, lines 3-10). Thus the use of his method to deliver tumor-specific antigens or therapeutic agents synthetic genes would be indicated by cancer. The term "anti-cancer agent" is not defined in the specification. Without a definition, using the broadest

reasonable interpretation, the term "anti-cancer agent" is being defined as "an agent which mitigates or treats cancer." One of the antigens Powell teaches is the expression of tumor-specific antigens, such as PSA, TAG-72, CEA and MAGE-1 may help "clear malignant tumor cells displaying the same antigen (column 18, lines 11-20), thereby having an anti-cancer effect. Thus the method would be directed on a tumor cell expressing the same antigen.

40. Powell teaches that the preferred animal cells of his inventions are human cells and cell lines, and that the cells may be present in the intact animal (column 7, lines 59-column 11 line 7).

41. Thus Powell teaches a method of creating an immune response (physiological response) comprising the step of introducing a foreign antigenic agent (therapeutic agent) into the cell of a human by contacting the cell with an non-virulent (attenuated) *E. coli* bacterium comprising a first gene encoding a secreted foreign functional cytolysin operably linked to a heterologous promoter, and a second gene encoding the foreign antigenic agent under conditions whereby the agent enters the cell, wherein an immune response (physiological response) is generated, wherein the method is indicated by cancer, and the therapeutic agent/foreign antigenic agent is an anti-cancer agent.

42. A skilled artisan would have been motivated to combine the teaching of Darji on a method of generating an immune response comprising the step of introducing a foreign antigenic agent (therapeutic agent) into a cell by contacting the cell with an attenuated (non-virulent) bacterium comprising a first gene encoding a nonsecreted listeriolysin capable of creating pore-forming holes in the attenuated bacteria and the

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vacuole of the cell, operably linked to a heterologous CMV promoter, inherently capable of driving expression of the listeriolysin in the bacterium, and a second gene encoding a foreign antigenic agent that is different that the nonsecreted listeriolysin, ActA, under conditions whereby the agent enters the cell and an immune response is generated further with the teaching of Powell on a non-virulent *E. coli* engineered to express a secreted cytolysin for the purposes of treating cancer using anti-cancer antigens in humans, because while Darji teaches that his method is performed in mice, he states that the use of attenuated bacteria for vaccination purposes in humans is well known, thus the skilled artisan would have been motivated to combine the teaching of Darji with the teaching of Powell to introduce the foreign antigenic agents (therapeutic agents) into human cells and humans as taught by Powell as a way of fulfilling the goal of treating humans and broadening the therapy options of the method to treat cancer. It would have been obvious to the skilled artisan to combine the teaching of Darji et al on a method of generating an immune response (physiological response) comprising the step of introducing a foreign antigenic agent (therapeutic agent) into a cell by contacting the cell with an attenuated (non-virulent) bacterium comprising a first gene encoding a nonsecreted listeriolysin capable of creating pore-forming holes in the attenuated bacteria and the vacuole of the cell, operably linked to a heterologous CMV promoter, inherently capable of driving expression of the listeriolysin in the bacterium, and a second gene encoding a foreign antigenic agent that is different that the nonsecreted listeriolysin, ActA, under conditions whereby the agent enters the cell and an immune response is generated further with the teaching of Powell on a non-virulent *E. coli*

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engineered to express a secreted cytolysin for the purposes of treating cancer using anti-cancer antigens in humans because using the non-secreted cytolysin created by Darji in humans is the ultimate goal of the development of attenuated bacterial vaccines, as specifically taught by Powell but also using the methods of Powell on bacterial vaccines as anti-cancer agents broadens the scope and function of the bacterial vaccine developed by Darji, thereby adding more therapeutic uses for the methods developed by Darji. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the instant invention was made, it must be considered that said ordinary skilled artisan would have had reasonable expectation of success in practicing the claimed invention.

Conclusion

43. No claims are allowed.

44. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kimberly A. Makar, Ph.D. whose telephone number is 571-272-4139. The examiner can normally be reached on 8AM - 4:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Irem Yucel, Ph.D., J.D. can be reached on (571) 272-0781. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Kam/03/11/07


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PRIMARY EXAMINER